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ORIGINAL ARTICLES

Outbreak report

OUTBREAK OF HUMAN LISTERIOSIS ASSOCIATED WITH TOMME CHEESE IN NORTHWEST SWITZERLAND, 2005

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During an eight week period in spring 2005, 10 cases of listeriosis were reported in a small area of northwest Switzerland (150 000 inhabitants). Eight cases were in older immunocompromised patients who became ill with bacteraemia (three deaths), and two cases were in pregnant women who had septic abortion. All cases were due to a serotype 1/2a isolate with one of two pulsovars found by PFGE. Patient interviews quickly revealed that a locally made and distributed soft cheese (known as 'tomme') was the food source responsible for the outbreak. Samples of this cheese, and of butter made in the same factory, revealed *Listeria monocytogenes* sv 1/2a of the same pulsovar in amounts of 1000-10000 and 10-100 cfu/g, respectively. The prompt suspension of production, the market recall of the product, and a public alert terminated the outbreak. However, two cases of febrile gastroenteritis due to the same strains were reported within 10 days of product recall. The restricted distribution area of the contaminated cheese and the collaboration of local physicians, medical microbiologists and food health services all contributed to a rapid and successful investigation.

This small outbreak of listeriosis reinforces the need for a laboratory-based surveillance system with rapid typing, as well as collaboration between physicians and microbiologists.

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Introduction

Human listeriosis is endemic in Europe, with an annual incidence varying between 0.3 and 0.7 cases per 100 000 inhabitants [1]. It has only been 25 years since the recognition that human listeriosis is almost exclusively a foodborne disease, and in this time, many outbreaks of varying extent have been reported, mostly in Europe and North America. The food items most often implicated in

outbreaks have been dairy products (milk, soft cheese), meat (paté, rillettes, sausage and various delicatessen), fish (smoked trout), and vegetables (coleslaw, sweetcorn salad) [2].

Between 1983 and 1987, Switzerland experienced a long-lasting outbreak of listeriosis due to the contamination of a locally produced soft cheese, causing at least 122 cases, of which 31 were fatal [3]. As a consequence of this outbreak, the federal health authorities (Swiss Federal Office of Public Health, SFOPH) designated a National Reference Centre for Listeriosis (CNRL), one of the tasks of which is to collect and characterise *L. monocytogenes* isolates, primarily from humans, but also from animal, food and environmental samples taken in Switzerland. The CNRL operates in close cooperation with the clinical microbiology laboratories and the cantonal (regional) laboratories responsible for environmental surveillance and food safety. The report of culture-confirmed human cases of listeriosis to the SFOPH and the sending of isolates to the CNRL have been mandatory for laboratories in Switzerland since 1988.

Between 1990 and 2005, the annual number of culture confirmed cases of human listeriosis has varied between 14 (in 1990) and 70 (in 2005), corresponding to 0.14 and 0.9/100 000 inhabitants per year [4]. During this time period, the proportions of bacteraemia (40%), central nervous system (CNS) infections (40%), and materno-fetal infections (20%) remained relatively constant.

Methods

The laboratory surveillance consists of confirming the identification of the isolates to the species level and typing the *L. monocytogenes* isolates. Serotyping is carried out as a first step screening method using a commercial agglutination test (Denka Seiken, Tokyo, Japan) based on antibodies specifically reacting with somatic (O) and flagellar (H) antigens.

This step is completed with pulsed field gel electrophoresis (PFGE) if a cluster of isolates is observed, based on geographic consideration, multiple cases on a short period of time, or cluster of isolates with identical serotype. PFGE was done following the PulseNet protocol (PFGE after DNA digestion with the enzymes Apa I and Asc I) (<http://www.pulsenet-europe.org>).

Interviews with patients and analysis of milk products were conducted by the local food authorities, the regional chemistry laboratory (Service de la consommation, Neuchâtel). Patient interviews were carried out face to face or by phone by a specialist microbiologist from the regional laboratory.

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L. monocytogenes in milk and milk products was detected according to the official methods in the Swiss Food Manual (http://www.bag.admin.ch/slmb/aktuell/d/56_Mikrobiologie.pdf). The method is based on an enrichment step, followed by plating on a selective agar and confirmation tests. The method for quantitative detection of *L. monocytogenes* on ALOA-agar was also used.

Results

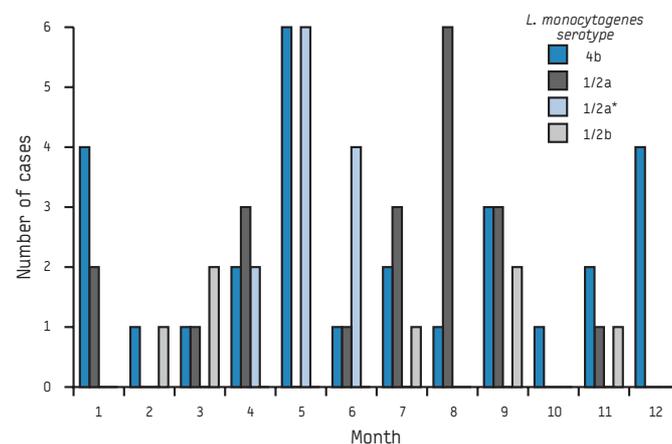
Within a period of 7 weeks in spring 2005, 10 human cases of listeriosis were diagnosed in a small area of northwest Switzerland (the canton of Neuchâtel, 150 000 inhabitants) by local physicians and clinical microbiologists. These 10 patients were admitted to three different hospitals [TABLE]. A single clinical microbiology laboratory serves these three hospitals and documented all 10 cases microbiologically. Four of the cases were in men (age range 70-72 years) and six were in women (two pregnant women, ranging in age between 23-26 years; and four non-pregnant women, aged 59-82 years). Clinical manifestations were bacteraemia confirmed. No CNS manifestation occurred. Seven patients had a severe underlying disease or condition; three died within 30 days of admission to hospital, and both pregnancies ended in septic abortion. Two further adult patients living in neighbouring cantons were diagnosed with listeriosis with febrile gastrointestinal symptoms during the following two weeks.

All 12 isolates (10 invasive isolates and two isolates from stool samples) belonged to serotype 1/2a, which has been the most commonly reported serotype in human and food isolates in Switzerland since 1995 [4]. Before this outbreak, however, only 3/18 (17%) human isolates reported in 2005 were of serotype 1/2a [FIGURE], which led the outbreak investigators to suspect a common source. For this reason, and because of the unusually high number of human cases of listeriosis recorded in a short period of time, the physicians and clinical microbiologists involved suspected a common source of infection, and contacted the regional chemistry laboratory (Service de la consommation) in Neuchâtel, which also has responsibility for food safety in the area. On 4 June 2005, a microbiologist at this laboratory interviewed five patients face to face in hospital or at home, and conducted a telephone interview

with a sixth patient. The interviews strongly suggested that a locally produced and distributed soft cheese known as a 'tomme' could be the origin of infections. Only one cheese factory in the region produced tomme cheese. Five samples of the suspected cheese were taken from the factory on 6 June and analysed immediately.

FIGURE

Serotype distribution of human cases of listeriosis registered, Switzerland, 1 January-31 December, 2005



* Tommes-related

Swiss legislation decrees a limit for *L. monocytogenes* which is 'not detectable in 25g', and the regional laboratory analyses milk and milk products according to these criteria. In addition to that, the laboratory applied the method for quantitative detection of *L. monocytogenes* to gather information about the average *L. monocytogenes* counts in the suspected cheese. It should be noted that the method for quantitative detection of *L. monocytogenes* on ALOA-agar gives results faster than the presence-absence test in 25g of food, which takes between three and four days to complete. In the quantitative detection method, ALOA-agar plates are incubated at 37 °C for 24 to 48 hours. Using this method, one of the five cheese samples was found to be positive for *L. monocytogenes* the next day (7 June).

TABLE

***L. monocytogenes* (serotype 1/2a) outbreak related to the consumption of Tomme cheese. Patient characteristics, Switzerland, 2005**

Case number	Sex	Age (years)	Date of isolation	Site	Hospital	Underlying disease or condition	Outcome	PFGE type (Apa I, Asc I)
1	M	72	18 April 2005	Blood	1	Myeloma		A
2	M	72	21 April 2005	Blood	2	Renal transplant		A
3	M	72	1 May 2005	Blood	1	Myeloma	Death	B
4	F	77	20 May 2005	Blood	2	Renal transplant		A
5	M	70	20 May 2005	Blood	3	Renal cancer	Death	B
6	F	80	27 May 2005	Blood	2	Renal dialysis		A
7	F	59	27 May 2005	Blood	1	Immunosuppressive drug	Death	A
8	F	26	31 May 2005	Blood	3		Septic abortion (22 w*)	A
9	F	23	1 June 2005	Placenta	1		Septic abortion (15 w*)	A
10	F	82	5 June 2005	Blood, stool	1			A
11	M	53	7 June 2005	Stool	-	Febrile gastroenteritis		A
12	F	47	17 June 2005	Stool	-	Febrile gastroenteritis		A

* Weeks of gestation

After enrichment, three of the five samples were found to be positive. In the following days, the laboratory analysed several more cheese samples which were delivered by consumers after the recall of the product and the public alert. These samples were all found to be positive for *L. monocytogenes*.

After PFGE analysis with 2 restriction enzymes (Apa I and Asc I), the 12 human isolates, and 14 food and food environmental isolates, were found to be of two pulsovars [6]. Ten human isolates and 12 food isolates shared outbreak pulsovar A, and two human and 2 food isolates shared outbreak pulsovar B [TABLE]. Among 29 other serovar 1/2a *L. monocytogenes* isolates recovered during 2004 and 2005 that had been tested for pulsovars, none shared the pulsovars A or B. The PFGE subtyping clearly confirmed the epidemiological link between the incriminated cheese and 10 cases of invasive listeriosis. It is interesting to note that the 1983-87 Swiss outbreak was also caused by two different strains [3].

A large national retailer sold butter in this region that was produced in the incriminated cheese factory (this butter was only sold within the local area). When the outbreak was first reported, this company analysed unopened samples of butter in their own laboratories. Five out of 10 samples from two different lots were found to contain *L. monocytogenes* of serotype 1/2a in 25g, and therefore, according to legislation, could not be sold. The bacterial counts in the five samples were lower than 100 cfu/g, which is the detection limit of the method for quantitative analysis of *L. monocytogenes*. These findings were later confirmed by the regional laboratory. Up to 32 000 cfu/g of *L. monocytogenes* were found in the tomme cheese, a level of contamination significantly higher than that in the butter.

Since the interviews with the listeriosis patients clearly pointed to tomme cheese from a particular producer, risk management measures were taken before bacteriological results were available. Production of the suspected tomme was suspended, and cheeses sold under one particular brand name were recalled from the market, and a public alert and press information were released on 6 June. On 7 June, after 24 hours of incubation, one of five cheese samples showed presumptive colonies on ALOA-agar and thus confirmed the need for the measures that had been taken the day before. Furthermore, a legally binding order was issued to the management of the cheese factory by the national authorities, asking the factory to perform environmental analyses in order to identify the weak points in the production process that had caused the outbreak. These investigations were done by microbiologists from Agroscope-Liebefeld (formerly the Swiss Dairy Research Station). It was demonstrated that *L. monocytogenes* was widespread throughout the facilities, but it was not possible to discover where the incriminated

L. monocytogenes strains had originated. At the time of writing this paper, the cheese factory had not yet restarted production, although the required sanitary measures had been taken.

Discussion

Swiss food legislation decrees a microbiological criterion for *L. monocytogenes* in milk and milk products which is 'not detectable in 25 g'. In the Neuchâtel outbreak, both tomme cheese and butter were found to exceed this limit and were therefore not acceptable under the current legislation. The EU regulation on microbiological criteria for foodstuffs, which will be incorporated into Swiss food legislation in the near future, differentiates between ready-to-eat food where *L. monocytogenes* can grow, and those foods where further growth is not likely. For the first group of foods, *L. monocytogenes* must be 'absent from 25 g', and for the second group, *L. monocytogenes* must not exceed 100 cfu/g. It is not clear to us how the EU regulation should be interpreted with regard to *L. monocytogenes* in butter. According to the findings of a Finnish study. [5], it is possible for *L. monocytogenes* to grow in butter. For this reason, we think that butter also should comply with the requirement to have *L. monocytogenes* 'absent in 25 g'.

The availability of a laboratory-based surveillance system with rapid typing, and the early raising of suspicion by local medical and microbiological staff, allowed rapid investigation of this outbreak and rapid recognition of the source. Considering the international distribution of many foods that may be a high risk for listeriosis infection, this illustrates the utility of an international surveillance network such as the one currently in development [7].

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